Chapter 10
Marine Sponges for Bioremediation Purposes and for Secondary Metabolites Production

Ismail Marzuki and Khairun Nisaa

Abstract Environmental bioremediation is necessary to maintain the balance of the ecosystem to remain friendly and supports the continuity of life in the future. Comprehensive screening of marine sponges, symbiotic bacteria and secondary metabolites produced has been carried out. The activity begins with the identification and characterization of the morphology and histology of sponges. Furthermore, the analysis of phenotype and genotype of symbiotic bacteria continued by exploring the function of several types of bacteria in the biodegradation method of PAHs and bio-adsorption for several kinds of heavy metals. These activities include analyzing secondary metabolite components produced by sponges with specific characteristics and specific behaviour of enzymes in enzymatic reaction mechanisms in several environmental improvement uses. Based on the screening results, it is known that there are 11 types of marine sponges from Kodingareng Keke Island, which are in symbiosis with eight varieties of bacteria from the Bacillus sp., Pseudomonas sp. and Acinetobacter sp. groups. These bacteria can biodegrade PAHs, especially against petroleum sludge, naphthalene and pyrene. Also found 12 types of symbiotic sponge bacteria with the ability to bio-adsorb heavy metals, especially Cr (III), Cr (VI), Mn (II), Mn (VII), Pb, Hg, As, Cu and Ni. Adsorption varies. The interesting part of the results of the bacterial symbiotic test was that three types of symbiotic sponge bacteria were found, which have dual functions as biodegradators of PAHs and bio-adsorbents of heavy metals. The sponges included *Acinetobacter calcoaceticus* strain PHCDB14, *Bacillus pumilus* strain GLB197 and *Pseudomonas stutzeri* strain SLG510A3–8. Therefore, this type of sea sponge is recommended for population propagation through the transplant method.

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10.1 Introduction

Exploration of marine microorganisms to overcome environmental pollution is essential to maintain and maintain the existence of the earth as the best and most comfortable dwelling for every living creature, more specifically in the marine environment, which is an area where various types of ecosystems of life and activities of animals to maintain themselves exist, on earth. It is realized that the actions and dynamics of human life contribute the most in producing various environmental problems today. Environmental pollution in multiple parts of the earth, the largest, is caused by human activities in maintaining their lives, directly and indirectly harming environmental sustainability (Dadrasnia et al. 2020). Human initiatives and efforts to save the environment are not merely desires that are free from interests and without purpose but are indirect efforts to maintain their existence on earth as living beings who want to exist and live for all time (Idah et al. 2018).

Human efforts to save the environment are carried out in various ways. These methods are, for example, by conducting research, experiments and trials both on a micro-scale and on a broad scale, whether carried out in the laboratory or directly carried out in the field. These activities become a necessity of life that humans must carry out because humans are most responsible and have an interest in maintaining the environment to remain a friendly place. Seeing that the living environment of living beings is not simple, comprehensive studies and studies are needed so that the environment remains sustainable throughout time so that the natural balance and dynamics of the ecosystem continue to run naturally (Marzuki et al. 2020a). Actual examples of human activities that contribute to the contaminant component for the environment can be seen in the petroleum mining industry, which produces various types of fossil fuels to meet the needs of life (Lu et al. 2019). There are three main activity stages of petroleum production, namely the stages of production through the processing industry, distribution and use of the product. The processing stage turns out that petroleum exploitation activities always produce by-products in the form of oil sludge or sludge. The distribution stage, which generally uses sea transportation in the form of tankers and distribution pipes, has the potential for incidents of distribution pipe leaks, tanker fires, collisions, sinking ships and other potential incidents that result in oil spills and pollute the environment (Medic et al. 2020). The stage of using this fuel to run production machines, cars, motorcycles and other work equipment that uses fossil fuels produces various types of combustion gases, all of which put a burden on the earth or the potential for environmental pollution (Rua et al. 2018).

Petroleum sludge is composed of two main components, namely hydrocarbon components and heavy metals. Hydrocarbon components contain several sub-components: total solid residue, fixed residue, volatile solid residue and sludge moisture content. These components are suspected of having hazardous and toxic materials such as polycyclic aromatic hydrocarbons (PAHs), both simple ones such as benzene to heavy ones such as benzo(a)pyrene (Marzuki et al. 2021c). Several types of heavy metals are often found in petroleum sludge, such as lead (Pb), nickel (Ni), copper (Cu), arsenic (As), chromium (Cr), aluminium (Al) and other types of heavy metals. Based on the characteristics of the components of PAHs and heavy metals that make up petroleum sludge, we can state that these components are a severe threat to the air, water and soil environment, so they must be watched out for and prevented from contaminating the environment (Marzuki et al. 2020b; Lajayer et al. 2019). We can find pAHs components in the form of gaseous residues combined with air, liquid, solid and semi-solid. Heavy metal components also include toxic elements to plants, animals and humans (Marzuki et al. 2020c). Generally in the form of particulates that can mix with air, associate with water and soil. Observing the characteristics of PAHs and heavy metals that make up petroleum sludge, We can say that these components are a severe threat to the air, water and soil environment, so they must be watched out for and prevented from contaminating the environment (Yogaswara 2017).

The sea area is a giant container, is the last shelter of the processes that occur in nature. The ocean is also a place for recovery to take home to a balanced state that occurs naturally due to shifts in a balance due to the dynamics of living things that appear on the earth's surface. Pollution that occurs in the atmosphere by heavy metal particulates and other contaminants will eventually end up in the oceans. The same is true for soil contamination by PAHs, heavy metals, microplastics and other components. Pollutants over time, the occurrence of rain that flows following low areas and finally empties into the sea will eventually empty into the ocean (Ziarati et al. 2019a, 2019b; Tereza et al. 2018).

The sea has protected itself to recover the presence of foreign contaminants through natural processes in which dynamics occur, traversed by adaptation between components until the balance is achieved. The problems that exist are contaminants that enter the sea body with the characteristics: (1) large volume, (2) various types, (3) toxic, (4) varying or even non-biodegradable decomposition time. The characteristics of these contaminants are variables that contribute dominantly to the recovery that occurs in the sea. These four variables tend to increase over time so that at a particular time, they pass the tolerance threshold for recovery that happens at sea. This condition causes the capacity and ability, performance and degradability of materials in the sea to decrease. The range of balance that can provide continuity of life is getting wider (Marzuki et al. 2021d).

The effect of hazardous materials in an area creates a chain pressure on various aspects of life, not only on the habitat of a population but also harms environmental productivity in providing support for long-term survival. Of course, this condition cannot be left alone. Herefore, we need solutions and concrete steps to reduce the volume and types of contaminants. In addition, we also need efforts to increase the carrying capacity of the environment for the lives of living things, especially humans, so that they remain in a balanced position between the needs of humans and living things with the availability and production of all conditions that can be provided by the environment (Parhamfar et al. 2020). Environmental management is determined by technological advances and environmental engineering, either by physical, chemical or biological methods. Aspects of utilizing the wealth of natural materials, both plants and animals, including microorganisms, have a decisive role in environmental management (Orani et al. 2018).

Various types of living things, including plants, animals and microorganisms, can potentially use pollution management and environmental management, which are available and widely distributed both inland areas and in the aquatic environment. Several groups of microorganisms such as fungi, fungi and bacteria are commonly used in environmental improvements to overcome and control the toxic nature of pollutant contaminants. The hydrocarbon, heavy metal and microplastic pollutant components are most often found accumulating in waters, especially the sea. In the marine environment, it is also found that many types of microorganisms have the potential to be used as materials in improving environmental quality (de Kluijver et al. 2021). Bioremediation methods to enhance the quality of the environment contaminated with toxic components using microorganisms have been widely applied. These activities are carried out both on a small scale in the laboratory, as well as on a medium scale and continuously by the company internally, especially in managing the waste produced, so that it meets the requirements for disposal to the accessible environment (Knobloch et al. 2018).

Marine sponge is a marine biota used as a biomonitoring material to determine heavy metal pollution in the marine environment. Determination of the pollution level is carried out by slicing certain body parts of the sponge as a sample. Then preparation is carried out so that the type and content of heavy metals in the sponge can be measured using ICP and or SSA instruments (Akinde and Iwuozor 2012). The measurement data obtained can be used as a basis for determining the level of heavy metal contamination in the waters of the sponge habitat. This example shows that sea sponges are bioindicators of heavy metal pollution. The results showed that several types of sponges could survive in aquatic environments contaminated with heavy metals, presumably due to the ability of these sponges to symbiotic with certain types of bacteria and force them to produce substances that behave enzymes. The substance is then spread on the surface of the sponge body as a mask to protect itself from the stress of heavy metal toxicity (Marzuki et al. 2020b).

Further exploration of sponges, especially their way of life, growth, breeding, self-defence against predators, and their adaptability to the presence of contaminants, is an interesting study to analyze because it holds many secrets of knowledge that need to be learned to be solved. This study also includes a unique feature of sponges: they can associate and symbiotically with several types of microorganisms. In addition, sponges also can nourish the hydrocarbon components. Sponges also play a role in forming parts of metabolically active substances by linking to the ability of environmental bioremediation through biodegradation of hydrocarbon components and bio-adsorption of heavy metals (Schmittmann et al. 2020).

10.2 Potential and Contribution of Marine Sponges in Environmental Bioremediation

Sponges are one of the marine biotas widely used as biomaterials to evaluate and analyze the presence of harmful contaminants in the marine environment. The role of sponges as biomonitoring and bioindicators of the quality of the marine environment is interesting to be studied more systematically. Many aspects need to be analyzed against marine sponges, starting from the way of life, nutrition, growth and symbiotic ability with microorganisms concerning the function of biomonitoring and bioindicators of pollutants and the level of pollution that occurs in the marine environment. A lot of evidence shows that some types of marine sponges can survive and even breed in environments contaminated with toxic wastes, such as hydrocarbon compounds, especially PAHs, heavy metals and microplastics (Marzuki et al. 2021a). The phenomenon of adaptation of sponges to the environment polluted with toxic components gives several assumptions: first, the body of the sponge can carry out the function of detoxifying contaminants; second, sponges can produce mucus substances that are spread on the body surface to protect the penetration of toxic materials; third, marine sponges *Auletta sp., Clathria (Thalysias) reinwardtii*, *Callyspongia aerizusa* are biomonitoring and bioindicators of heavy metal pollution levels (Marzuki et al. 2021b); Fourth, several types of marine sponge symbiotic bacteria of the Bacillus group (*Bacillus licheniformis* strain ATCC 9789, *Bacillus sp*. partial AB353F) and Pseudomonas (*Pseudomonas stutzeri* RCH2, *Pseudomonas stutzeri* strain SLG510A3-8) were able to degrade hydrocarbon components (Marzuki et al. 2020a; Orani et al., 2020a; Orani et al. 2018).

The world's population of sponges is estimated at 15,000 species scattered in seas and lakes, but only 46.67% or ±7000 species have been reported, and only 830 species have been isolated and characterized. Sponges are living organisms with a reasonably old civilization that has existed since ±600 million years ago. Sponges can associate with many different microorganisms, including cyanobacteria, heterotrophic bacteria and unicellular algae (Campana et al. 2021a). Sponges are primitive multicellular animals (metazoans) without natural tissues, unique ways of life, capturing food by filter feeders. Sponges are sponges that reproduce both asexually and sexually. Asexual reproduction occurs by the formation of internal buds or gemmules (Maldonado et al. 2021). Sponges can produce and contain more active compounds than compounds produced by land plants. The facts attached to sponges are an essential and logical argument as multi-functional animals in the role of biota upholding the balance of the ecosystem (Schuster et al. 2018).

Bioindicators of heavy metal pollution in the sea can be determined by analyzing the growth and development of sponges (Siahaya et al. 2013). Several research results show that marine sponges contaminated with several heavy metals do not experience growth and development disorders (Melawaty et al. 2014). These conditions indicate that marine sponges can adapt to the presence of heavy metal contaminants in their habitat. The method of determining heavy metal contaminants in the marine environment is carried out by analyzing the types of heavy metals in the sponge's body. Meanwhile, the concentration level of heavy metal contamination in the marine environment is carried out using appropriate instruments, for example, analysis using Atomic Absorption Spectrophotometer (SSA). It can also be carried out using Inductively Coupled Plasma, both optical emission type (ICP-OES) and mass spectrometry type (ICP-MS) (Ulli et al. 2016).

Sea sponge, one of the biomaterials for monitoring heavy metal pollution in the marine environment, is an essential contributor to marine natural materials in maintaining the balance and sustainability of the growth and development of ecosystems in the sea. Sponges also act as biodegradators of contaminants containing hydrocarbon components. Sponges also act as biodegradators of contaminants containing hydrocarbon components. Symbiotic bacteria of marine sponges play a role in the biodegradation of polycyclic aromatic hydrocarbons (PAHs) (Marzuki et al. 2021c, d). Pyrene, anthracene, phenanthrene and naphthalene are PAHs that symbiotic sponge bacteria can degrade. Types of PAHs-degrading sponge symbiont bacteria include *Bacillus licheniformis* strain ATCC 9789, a sponge symbiont of *Auletta Sp*; *Acinetobacter calcoaceticus* strain PHCDB14, symbiont *Callyspongia aerizusa*; *Bacillus Sp* isolated from Neopetrosia Sp; *Bacillus pumilus* strain GLB197 isolate sponge *Niphates Sp*; and *Pseudomonas stutzeri* strain SLG510A3-8 isolate the sponge *Hyrtios erectus* (Marzuki et al. 2020b). Bacteria Bacillus sp. AB353f partial symbiosis of Neopetrosia sp. *Bacillus cohnii* strain DSM 6307 symbiont Niphates sp. Several types of sponges suspected to have potential symbiotic bacteria in the biodegradation of PAHs based on the results of identification and morphological characterization include Petrosia (Strongylo Phora) corticata, Clathria (Thalysias) reinwardtii, Callyspongia sp., Coelocarteria singaporensis, Callyspongia (Cladocalina) vaginalis and Callyspongia (Cladocalina) vaginalis. Marzuki et al. 2020a). It was further explained that this type of sponge was thought to have potential symbiotic bacteria through histology of the sponge and analysis of the phenotype and genotype of the symbiotic bacteria. Researchers obtained all the types of sponges mentioned above, about 11 species from three different islands, namely Kodingareng Keke Island, Barrang Caddi Island and Langkawi Island, the administrative area of the Makassar City Government, South Sulawesi Province, Indonesia. These islands are included in the cluster of the Spermonde Archipelago (Marzuki et al. 2021d; Knobloch et al. 2018).

Sponges have an external morphological structure influenced by several general factors, both physical, chemical and biological habitats. Sponges that grow in different habitats have varying growth structures. Types of sponges that live in less stable, shallow, turbid and high waves water environments tend to have creeping growth and are generally shorter. In contrast, the same type of sponges that live in a more stable environment, such as protected growth areas, calm currents and deep waters, tend to grow taller, upright, more symmetrical and have a more extensive body posture (Marzuki et al. 2016; Laport et al. 2009).

The presence of predators, polluting contaminants and competition of sponges with other biota is thought to affect the morphology of sponge growth. The presence of predators such as echinoderms, prosobranchia, opisthobranchia and other types of predators affects the morphology of sponge growth, even forcing sponges to evolve body structure as a form of adaptation to avoid these predators. Several types of sponges have body structures to drill as a form of disguise and transformation to predatory threats (Yang et al. 2019).

Sponges that live in marine waters that contain coral and are overgrown with algae trigger a competition, where the sponge has a high chance of winning the match if all three are in a relatively deep environment and lack light. Still, the body structure of the growth of such sponges is generally angular (Costa et al. 2020). Sponges that live in marine waters contaminated with hydrocarbons, both aliphatic and aromatic groups, still have a high potential to survive and grow and develop because of their high adaptability to adapt through two mechanisms. First, the nutritional ability of sponges by eating matter. Organic suspended in water flow in body cavities and is sprayed back out. Second, Sponges can have a symbiotic relationship with certain microorganisms, especially bacteria that can selectively and specifically produce enzymes that behave in the form of mucus spread on the sponge body's surface. The function of the mucus is to protect and detoxify the toxic properties of PAHs (Kamaruddin et al. 2021). Sea sponges are also thought to have the ability to survive in areas contaminated with microplastics and produce mucus that can re-glue cracked concrete. Some of the benefits of this marine biota are that sponges deserve the nickname multi-functional biota and have been named green chemistry biota (Obire et al. 2020; Parama Cita et al. 2017).

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The internal structure of the marine sponge body at the cellular level is found in several parts such as oscula, surface granules, skeleton and spicules with varying skeletons from each type of sponge. A variety of skeletons are influenced by the growth environment and dynamics of life experienced by the sponge in its growth period. The growth dynamics of sea sponges are influenced by their growth habitat conditions, especially currents, depth, wave height, exposure to sunlight, nutrients, predators and contaminants. These factors also affect the structure and anatomy of cells. The histology of marine sponges based on the catalogue (Krishnamoorthy et al. 1983) is as per Fig. 10.1a–f, for the marine sponge *Niphates sp*., belongs to the family Niphatidae (Duchassaing de Fonbressin and Michelotti 1864).

The interaction mechanism of sponges with bacteria provides a place or sponge host for certain bacteria to carry out cell growth and division activities. Then there is interaction and adaptation to the new environment. If there are interfering components, then the host organism is stimulated to produce active substances or synthesize bioactive compounds as secondary metabolites. The active substance is delivered for self-protection and maintains environmental balance (Campana et al. 2021a, 2021b). The completion and arrangement of substances or chemical components of the active substance are natural. The type of active substance formed is

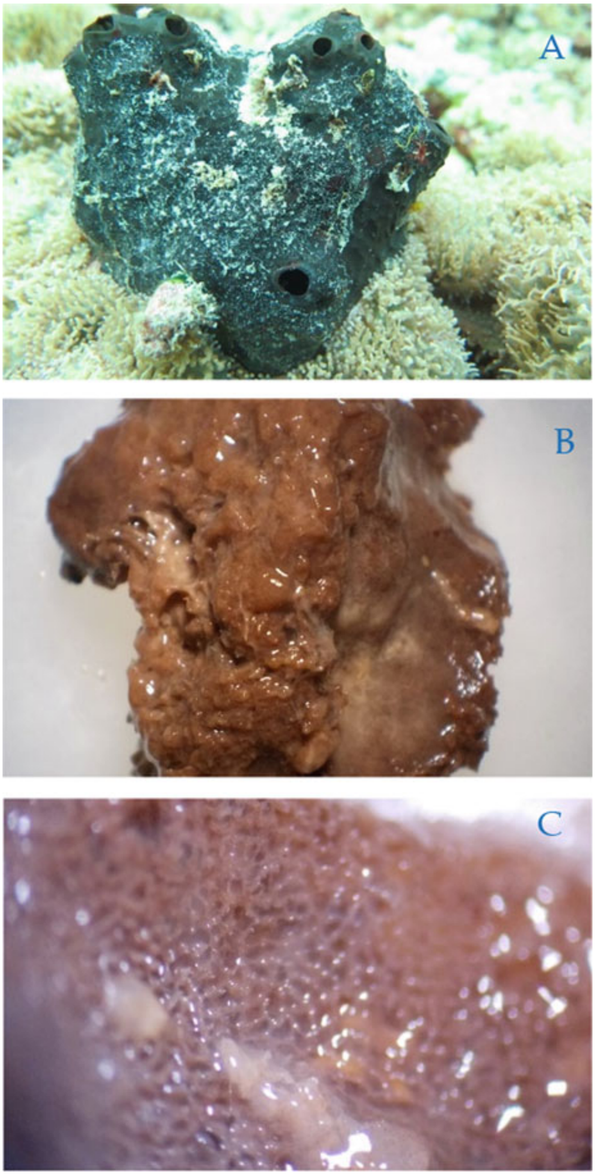


Fig. 10.1 (**a**) Growth from Sligthy globular sponge, with big size oscula. (**b**) Consistency Slippery surface sponge, covered by mudlike slime. Inelastic and brittle body sponge. (**c**) Surface granular sponge surface. (**d**) Skeleton Spicule skeleton with echinating spicule. (**e**) Skeleton tract Paucispicular tract skeleton with high fibre. (**f**) Spicule Small megasclera oxea (magnification 40x)

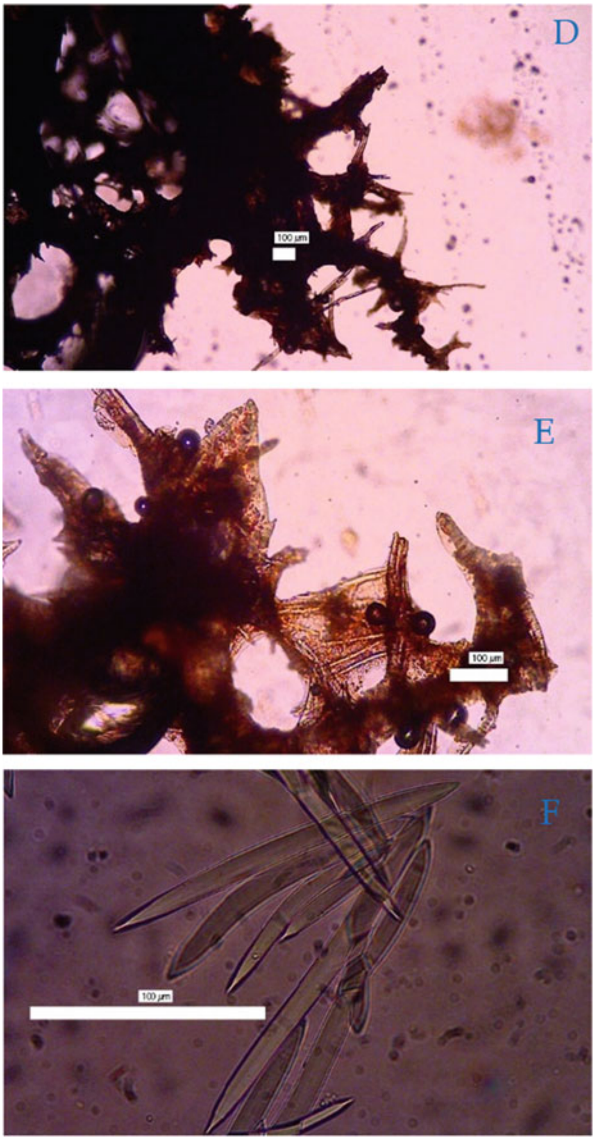


Fig. 10.1 (continued)

influenced by the kind of food and the interfering components in the sponge growth process. Generally, sponge nutrition is rich in new microorganisms with potential pharmacological activity so that sponges can carry out the growth process well, even in extreme environments. On the other hand, microorganisms or bacteria benefit from living on a sponge, i.e. bacteria are protected from the pressure of waves and currents. The interaction between sponges and bacteria occurs as a form of commensalism symbiosis, in which bioactive compounds are produced (Marzuki et al. 2020a).

Sponge symbiont bacteria have a significant influence on the formation of bioactive substances. This influence can be seen in the role of bacteria as the leading supplier of energy needed by sponges. Screening of symbiotic sponge bacteria was carried out by inoculating bacteria associated with the sponge on NA media overgrown with the test bacteria. This qualitative method can only determine whether the bacteria can inhibit the test bacteria E. coli and S. Aureus without knowing the effectiveness of these bacteria (Gunathilake 2018). It is estimated that 40% of the biomass of some types of sponges is composed of bacterial communities. Cyanobacteria symbiosis can make up one-third of the total mass of living tissue in some types of sponges. The energy needs of some types of sponges are supplied from 48% to 80% of microorganisms (Knobloch et al. 2018).

The bioremediation function of marine sponges on the environment is thought to be played by the active substances in the sponge's body. Sponges produce these chemical components to defend themselves against potential growth disorders. The characters, types and specific properties of these secondary metabolites are formed according to the dynamics of life experienced by sponges during their growth period. These metabolic substances are thought to have several functions and benefits, both in utilization studies in the medical field and environmental bioremediation processes (Obire et al. 2020). Its environmental remediation function is primarily for the application and function of biodegradation of PAHs and heavy metal bio-absorption, including the potential to overcome the presence of microplastics in the environment (Marzuki et al. 2021b). The researcher can achieve knowledge of the role and function of these sponges in the biodegradation of PAHs, bio-adsorption of heavy metals and the absorption potential of microplastic components by conducting a series of studies and analyses. The assessment and analysis activity begins with observing the sponge's growth environment, screening the morphology and histology of potential sponges for environmental remediation functions. The next activity is the isolation of symbiotic bacteria, characterization through phenotypic and genotypic analysis of symbiotic bacteria, to conducting micro-scale tests and field experiments to determine the performance of symbiotic sponge bacteria in the biodegradation of PAHs and bio-adsorption of heavy metals (Marzuki 2020; Rua et al. 2018).

10.3 Search for Potential Sponges for Bioremediation Functions in Polluted Environments

The method of determining potential sponges for the biodegradation function of PAHs was carried out by isolating potential sponge symbiotic microorganisms. The purpose of potential sponges is to select specific types of sponges that meet the following criteria: first, selecting sponges that are suspected of living in areas contaminated with hydrocarbons; second, the surface of the sponge body is covered by mucus or enzyme behaviour substances or at least dark-coloured sponges; third. The colour of the sponge is generally less bright. The selected sponges were then isolated to obtain bacterial isolates. Isolation of marine sponge symbiotic bacteria using a simple method, as shown in Experiment 10.1.

Experiment 10.1: Isolation of Marine Sponge Symbiotic Bacteria

The selected potential marine sponges were cleaned by spraying the surface using sterile seawater with a ratio of 1 cm2: 5 mL of sterile seawater. The mesohyl sections were ±1× 1 cm in size, crushed and diluted with sterile Phosphate Buffer Saline (PBS) in a ratio of 1:1. Isolate the bacteria on the outer surface using a sterile swab, then wipe in one direction on the outer surface of the sponge. The sterile swab was put into a dilution tube containing sterile PBS and vortexed. The results of the dilution were spread into a petri dish that already contained Seawater Complete (SWC) media, incubated at 26°C for 24–36 h, observed colony growth, bacterial morphology. Selected colonies, separated using a round needle, were purified using the same medium to obtain pure isolates. Purifying bacterial symbionts using the direct plating method was performed by scratching one ose of colonies in a zig-zag direction on a petri dish containing 100% marine-agar media. Incubation temperature 30°C, 1–2 days. Re-scratching on 100% marine agar until a single colony was obtained (Marzuki et al. 2021a).

Marine sponge symbiotic bacteria biodegraded PAHs contaminants by integrating one type (Naphthalene) with a potential bacterial suspension. The complete procedure for biodegradation of marine sponge symbiont bacteria against PAHs is presented according to the method in Experiment 10.2.

Experiment 10.2: Biodegradation of PAHs Using Marine Sponge Symbiotic Bacteria

Determination of potential bacteria is done through preliminary tests. Bacteria that pass the initial test are bacteria that show growth activity on hydrocarbon-contaminated media. Propagation of selected bacterial cells by culture. The bacterial suspension was made and determined the number of cells. Entered 25 mL of bacterial suspension in several degradation reactors (vials), adapted for 1 × 24 h, put 5 mL of contaminant PAHs (naphthalene) with a concentration of 1000 ppm, shaker incubator 200 rpm. Observations and measurements of media degradation parameters were carried out every 2 days for 30 days. Determination of the ability and performance of biodegradation of symbiotic bacteria were analyzed using Gas Chromatography–Mass Spectroscopy (GC–MS) instruments. Biodegradation products were analyzed using Fourier Transform – Infrared Spectroscopy (FT–IR) for serial interaction periods, such as 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 (Lu et al. 2019).

Parameters of PAHs biodegradation by symbiotic sponge bacteria consisted of optical density, pH, temperature, an abundance of gas bubbles and fermentation odour. Optical density was determined using a UV-Vis spectrometer on a mask. For each serial interaction period in multiples of 2 days. Suppose an increase in the optical density (OD) value for the first few days of interaction. In that case, it

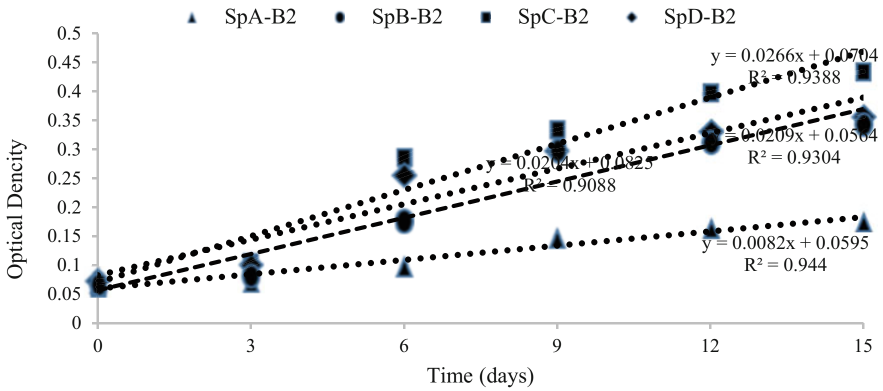


Fig. 10.2 The rate of increase in the value of OD (bacterial cell growth rate) based on the interaction time in days between a sponge symbiotic bacterial suspension and 10,000 ppm naphthalene. Source: Marzuki et al. 2021d)

indicates a bacterial activity in the degradation medium contaminated with PAHs until it enters the saturation period marked by no increase in the OD value in the next few days. The rate of increase in OD value can be identified with the growth rate of bacterial isolates isolated from four different types of marine sponges, namely *Hyrtios erectus* (SpA.B2), *Clathria (Thalysias) reinwardtii*) (SpB.B2), *Niphates sp.* (SpC.B2), and *Callyspongia sp.* (SpD.B2), respectively, interacting with 10,000 ppm naphthalene and 10,000 pyrenes for 15 days (Marzuki et al. 2021d), according to Figs. 10.2 and 10.3.

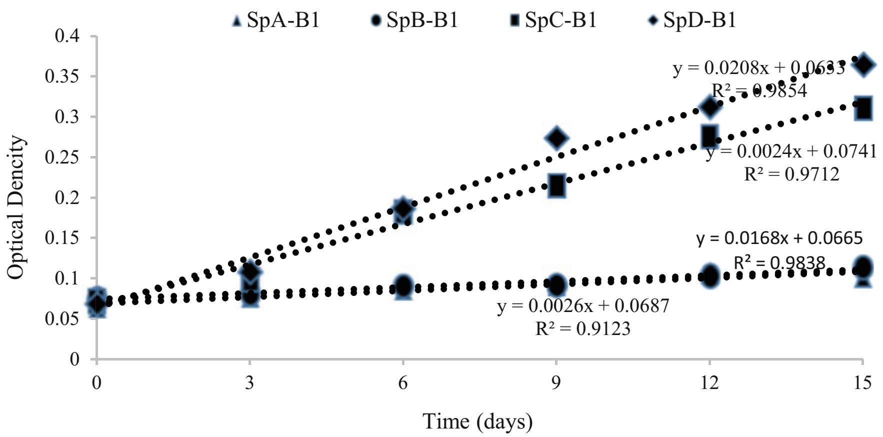


Fig. 10.3 The rate of increase in the value of OD (bacterial cell growth rate) based on the interaction time in days between a sponge symbiotic bacterial suspension and 10,000 ppm pyrene. Source: Marzuki et al. 2021d)

The increase in OD value in the reactor containing PAHs contaminants is directly proportional to interaction time. The rise in OD value indicated an activity of isolates of sponge symbiont bacteria containing PAH. These conditions showed that the bacteria were able to adapt to media containing PAHs contaminants, although the OD values of each isolate were different. The sequence of growth of symbiotic sponge bacterial cells in naphthalene-contaminated media (Fig. 10.2), SpC.B2 SpD. B2 SpB.B2 SpA.B2, while the growth rate of bacterial cells in pyrene-contaminated media (Fig. 10.3) in the order SpD.B2 SpC.B2 SpB.B2 SpA.B2.

Changes in the pH of the degradation medium towards a lower value or tend to be more acidic indicate that there is bacterial biodegradation activity on the hydrocarbon component substrate. The temperature of the degradation media tends to increase generally in the range of ±0.5–2.3 0C after the interaction lasts for several days if there is biodegradation activity in the reactor (Bendouz et al. 2017).

The gas bubbles in the reactor are relatively increased as a sign that there is biodegradation activity. The abundance of gas bubbles formed is directly proportional to the level of biodegradation. Gas bubbles are formed and generally increase after the interaction runs for a few days. The abundance decreases as the biodegradation process in the reactor weaken, indicating a decline in the number of bacterial cells that carry out the degradation process (Marzuki et al. 2020a). Gas bubbles formed as a sign that there are simple organic compounds in the reactor in methane gas, CO_2, NO_2 and other gases resulting from biodegradation or decomposition/ rehabilitation of PAHs components. The smell of fermentation is also part of the biodegradation parameter as an indicator. The degradation that occurs results from the work of bacteria through the mechanism of degradation of enzymatic reactions produced by biodegradative bacteria (Onwosi et al. 2017).

The speciality of bacteria in the PAHs degradation method is the ability of bacteria to destroy the hydrocarbon structure and turn it into an energy source for the activity and survival of bacteria. The biodegradation performance of symbiotic sponge bacteria against PAHs was determined using GC–MS. The data obtained on the chromatogram show the components of the biodegradation product (Marzuki et al. 2020c). The chromatogram data is in the form of component abundance peaks and the potential for new peaks to appear. In general, the abundance of the tested PAHs components as substrates will decrease with the emergence of new peaks. The higher the performance of bacterial biodegradation, the lower the peak height (abundance) of the PAHs test components. The lower the PAHs, the lower the concentration of the degradation components. Figure 10.4 shows an example of a GC–MS chromatogram resulting from the biodegradation of Bacillus SP strain AB353f in symbiosis with the Neopetrosia Sp sponge against naphthalene compounds.

The biodegradation process of PAHs (naphthalene) substrate at 1-day interaction was not seen (Fig. 10.4a). This biodegradation process began to appear after 10 days of interaction between Bacillus sp. strain AB353f and naphthalene (PAHs substrate). An indication between Bacillus sp. strain AB353f and PAHs substrate is indicated by the decreasing peak height of naphthalene in the graph (Fig. 10.4b). Decrease of peak showed that the abundance of the substrate had decreased, meaning that there

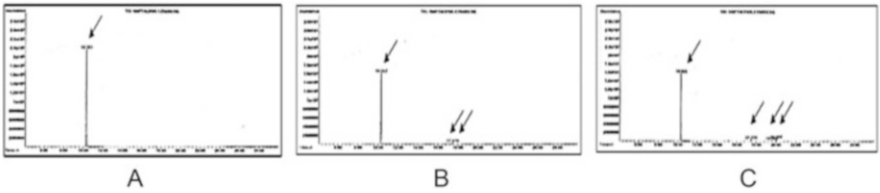


Fig. 10.4 Chromatogram of naphthalene biodegradation of Bacillus SP strain AB353f based on interaction time. (**a**). Interaction time 1 day; (**b**) The interaction time is 10 days, and C. the interaction time is 20 days (Marzuki et al. 2020c)

was a decrease in the concentration of PAHs along with the emergence of two new peaks resulting from biodegradation in the form of simple organic compounds. The new peak increased to three after the interaction period lasted 20 days (Fig. 10.4c), marked by the decreasing peak height of naphthalene which means that the biodegradation process continues. The interaction period in this experiment was up to 30 days. Still, it appears that during the interaction period above 20 days, there was no addition of new peaks, and the peak height of PAHs tended to stagnate. This condition indicates that the biodegradation process is no longer running. It could be because the bacterial cells as biodegradators died due to poisoning by the toxic nature of PAHs or because the bacterial cells could not withstand the increase in the acidic properties of the media in the reactor. The increase in the acidic properties of the reactor media occurred, presumably because components were resulting from the biodegradation of simple organic compounds from the carboxylic acid group (Marzuki et al. 2021c). The results of the GC–MS measurement also provide data on the level of biodegradation. After calculating, the result indicated that the biodegradation rate was in the range of 26% - 46% for using one type of symbiotic sponge bacteria against one type of PAHs (Costa et al. 2020).

Several ways can improve the biodegradation performance of bacteria against PAHs. For example, it increases the number of bacterial cells in the reactor, prolonging the life of bacterial cells by providing nutrients in adding nitrogen, phosphorus and potassium in the degradation reactor. In addition, during the interaction period, oxygen is supplied to prevent an increase in the acidity of the degradation media or the use of several types of bacteria (a consortium of bacteria), which can degrade hydrocarbon components. The use of consortium bacteria to increase the level of biodegradation does not necessarily increase the capacity of these bacteria to degrade, presumably due to competition between bacteria in obtaining energy supplies from the PAH components that have been destroyed. However, the biodegradation of PAHs with consortium bacteria is currently being carried out because of the ease of multiplying bacterial cells and can be carried out very quickly (Bendouz et al. 2017).

Many collections of bacteria that can biodegrade PAHs have been carried out, especially bacteria from the Bacillus and Pseudomonas groups. Both groups of these bacteria can be isolated from several sources, such as soil contaminated with PAHs, seawater contaminated with hydrocarbons, sponges and several other marine biotas.

A collection of PAH-degrading bacteria is known as hydrocarbonoclastic bacteria (Lu et al. 2019).

The mechanism of bacterial biodegradation of hydrocarbon components, both aliphatic and aromatic groups, in principle, is through the oxidation pathway to form alcohol. Alcohol products become aldehydes and finally produce carboxylic acids and a small portion of esters before entering the -oxidation cycle and producing energy for biodegradation activity—next cycle. The biodegradation process also produces by-products in simple organic compounds, such as CO2, methane gas, NO and other gases, depending on the degraded hydrocarbon component. The biodegradation of PAHs is relatively the same as aliphatic components (Medic et al. 2020). The striking difference for the aromatic hydrocarbon component at the stage after the oxidation reaction is that it is preceded by the destruction stage of the aromatic molecular structure so that the biodegradation of PAHs components by suitable specific bacteria takes longer. The resulting degradation rate is generally much smaller than in the degradation of aliphatic hydrocarbon components (Fang et al. 2020). A simple description of the biodegradation mechanism of aliphatic components is presented in Fig. 10.5.

Specific degradation pathways of Bacillus subtilis strain BAB-1684 sponge symbiont Callyspongia sp. against the hydrocarbon components of petroleum sludge waste after 20 days of contact are presented in Fig. 10.6.

Based on the illustration of the biodegradation of hydrocarbon components by the symbiotic marine sponge bacteria (Figs. 10.5 and 10.6), some steps allow the biodegradation process to stop completely. The biodegradation process stopped entirely because the bacterial cells experienced mass death. The biodegradation process can stop completely at the stage of changing the biodegradation products of aldehydes or ketones into carboxylic acids. The formation of carboxylic compounds can cause the interaction medium to become acidic so that bacterial cells have the potential to experience sudden death because they cannot survive at a certain acidity level in the degradation medium (Marzuki et al. 2021a; Yogaswara 2017). This stage is known as the reaction rate stop stage, or the biodegradation process stops completely.

The contribution of marine biota, especially sponges, in maintaining the balance of the marine ecosystem is substantial. The contribution of marine life, especially sponges, in maintaining the balance of the marine ecosystem is enormous. Suppose an in-depth review is carried out on the formation of secondary metabolite

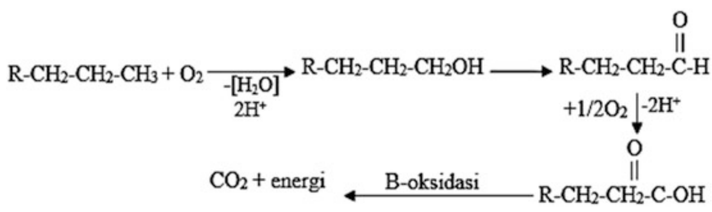


Fig. 10.5 Simple mechanism of biodegradation of aliphatic hydrocarbon components

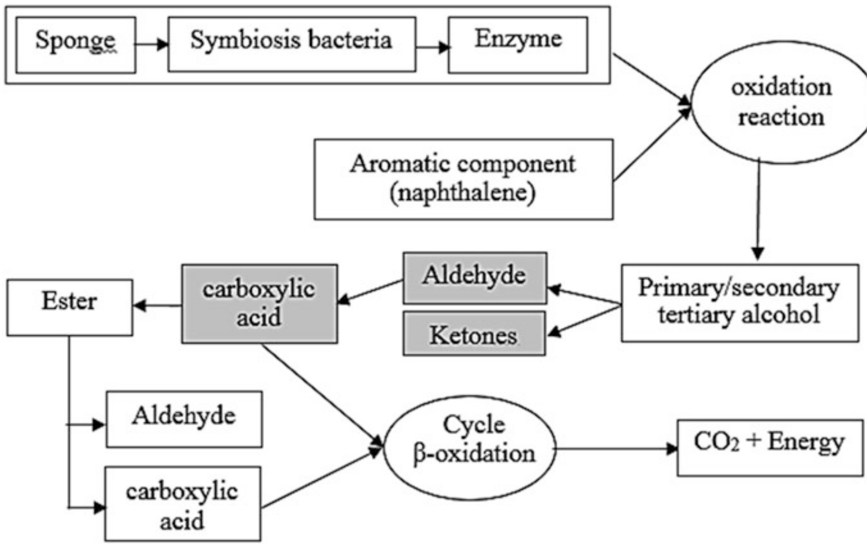


Fig. 10.6 Estimation of a simple path of biodegradation of aliphatic hydrocarbon components of petroleum sludge by the bacterium Bacillus subtilis strain BAB-1684 symbiosis of sponge Callyspongia sp

components of sponges and the effect of these substances on their biodegradability, bio-adsorption and activity of sponge bioactive substances against pathogenic bacteria and fungi. In addition, the active substance of sea sponges is thought to have the potential as primary and secondary raw materials for certain drugs to treat a disease (Shareef et al. 2016). Studies on sponges, symbiotic microorganisms and components of secondary metabolites are interesting to do more comprehensively, including studies related to the competition for the growth of sponges versus other biotas such as corals and algae if all three are in the same area (Schuster et al. 2018).

A simple study of the morphology and histology of marine sponges of the Niphates sp. species obtained from the sea waters around Kodingareng Keke Island has been described in point 10.2 above. Part of the characterization of sponges is to identify and characterize the symbiotic sponge bacteria that are thought to have biodegradation performance against PAHs potentially. Phenotype and genotype characterization of symbiotic sponge bacteria was carried out by taking samples of four isolates of sponge bacteria *Petrosia (Strongylo Phora) corticata* (Sp. 1), Auletta sp. (Sp. 2), *Neopetrosia sp.* (Sp. 3) and *Callyspongia aerizusa* (Sp. 4). (Marzuki et al. 2020a, 2020b, 2020c, Bioflux), are presented in Table 10.1.

The urease test results (Table 10.1) on two isolates (Sp. 1 and 2) showed positive results, meaning that both isolates were able to hydrolyze urea. This ability means that the isolate can produce and possess the enzyme urease. On the other hand, the isolates (Sp. 3 and 4) were negative, meaning that both isolates could not hydrolyze urea or did not have urease enzyme. V-P reagent test isolates (Sp. 1–3) showed a positive reaction, meaning that there were components in the three isolates capable of carrying out the fermentation reaction. In the methyl red test (R-Mr), the four

Table 10.1 Phenotype characterization of sponge symbiont bacteria Callyspongia sp, Biochemical Test method

Biochemical reagents	Media	Sponge bacterial symbiont			
		Sp. 1	Sp. 2	Sp. 3	Sp. 4
Starch hydrolysis	Starch agar	Base	Base	Base	Base
Casein hydrolysis	Milk agar	Acid	Acid	Acid	Acid
Gelatin hydrolysis	Gelatins	–	–	–	–
Nitrate reduction	Nitrate broth	–	–	–	–
Indole	Tryptone broth	–	–	–	–
H_2S	H_2S	–	–	–	–
Reagent methyl red	R-Mr broth	+	+	+	+
Reagent- Voges Proskauer	R-VP broth	+	+	+	–
Citrates	Citrate	+	–	+	–
Urease	Urea broth	+	+	–	–
Glucose	Glucose broth	–	–	–	–
Lactose	Lactose broth	+	+	+	+
Sukrose	Sucrose broth	–	–	–	–
Mannitol	Mannitol broth	–	–	–	–
Catalase	Nutrient agar (NA) slant	+	+	+	–

Note: + (reaction); – (no reaction)
Source: Marzuki et al. (2020a)

isolates showed a positive response, indicating that the isolates could produce acid in glucose fermentation (Marzuki et al. 2015). The catalase test on all isolates resulted in a positive reaction (Sp. 1–3 isolates). The isolates had catalase enzymes that could degrade hydrogen peroxide (H2O2), while the fermentation test used glucose reagent did not show any reaction activity. The lactose test showed that the four isolates gave a positive reaction. The sucrose test resulted in a negative response, meaning that the bacteria that grew in the fermented liquid media were acidic components (Bibi et al. 2016). The results of characterization through gram staining and biochemical tests showed that the two microsymbiont isolates of four different types of marine sponges contained enzymes and could ferment and process carbon from their environment. With the six criteria described above, it is suspected that three isolates (Sp. 1, Sp. 2, Sp. 3) have the potential and ability to degrade, especially to aliphatic hydrocarbon components. Still, it is necessary to test and follow up on analysis further to degrade aromatic hydrocarbons (Khabouchi et al. 2020).

The genotype identification of four isolates from four different marine sponges was carried out to see the pair structure and nitrogen base-pair composition through the Basic Local Alignment Search Tool (BLAST), PCR method. The results of the isolate sequencing were opened through the bioedit programme. The sample bacterial DNA sequences were entered into the BLAST (Basic Local Alignment Search Tool) programme (http://blast.ncbi.nlm.nih.gov/Blast.cgi), the sequences were identified with the DNA database. GenBank on the site (Kadhim et al. 2013; Cowden 1976). The results of the alignment of the sample sequences with the GenBank sequences showed a high similarity of the homologous series, which can be seen in the table as presented in Table 10.2.

Table 10.2 BLAST results of Symbiont Sponge bacteria, PCR method

Symbiont code	Sample Sequence	Sequence Gen Bank	Quantity (%)	Difference (%)	Species
Sp. 1	17–972 (955)	608.723–609.690 (967)	944/955 (98.85)	4/955 (0.42)	*Pseudomonas stutzeri* RCH2
Sp. 2	11–985 (974)	524.589–525.563 (974)	956/974 (98.15)	14/974 (0.01)	*Bacillus licheniformis* strain ATCC9789
Sp. 3	15–975 (960)	574.123–575.089 (966)	932/960 (97.49)	16/960 (1.66)	*Bacillus sp.* AB353F.Partial
Sp. 4	21–934 (913)	574.323–575.258 (935)	906/935 (96.90)	12/935 (1.28)	*Acinotobacter calcoaceticus* strain PHKDB14

Source: Marzuki et al. (2020a)

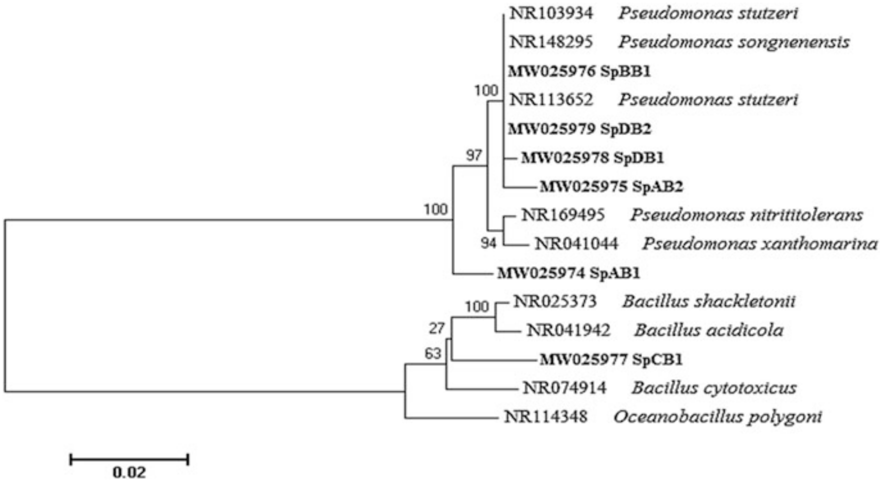


Fig. 10.7 Representative neighbour-joining tree reconstructed with the 16S rRNA sequences of marine sponge symbiont bacteria (Marzuki et al. 2021d)

The results of genetic analysis using PCR found each sponge symbiotic isolate (Table 10.2). There are two types of bacteria with different strains. Each of which is isolates from sponges Auletta sp. (Sp. 2), Neopetrosia sp. (Sp. 3), one group of Pseudomonas, isolates of sponges Auletta sp. (Sp. 2) and one group of Acinetobacter. Which is an isolate from sponge Callyspongia aerizusa (Sp. 4). Based on the results of biochemical tests (Table 10.1) by observing the reaction results of each test medium combined with the results of the identification of the 16S rRNA gene molecule by PCR method (Table 10.2). It is indicated that the four species can carry out chemical reactions to break down hydrocarbon molecules (Maldonado et al. 2021).

Characterization and phylogenetic identification of symbiotic sponge bacteria according to the reconstructed marine sponge symbiotic bacterial phylogenetic tree against 16S rRNA bacteria, three species were well resolved supported by moderate bootstrap values (Fig. 10.7). The first clade, isolates of sponges SpA.B2, SpB.B2, SpD.B2, live in groups with *Pseudomonas stutzeri* and *Pseudomonas songnenensis* with 97–99% homology. The second clade, consisting of SpA. B1/B2, which is positioned as a child clade of the Pseudomonas species. The third clade, SpC isolates. B1 grouped with Bacillus isolates reached 96%.

10.4 Development of the Function of Symbiotic Sponge Bacteria Through Heavy Metal Bio-Adsorption Method

The application of marine sponge symbiotic bacteria in bioremediation of heavy metal-contaminated environments is being developed. Several studies have shown that several types of bacteria can bio-adsorb heavy metals. Heavy metals such as Zn, Pb, and Cd can be absorbed using certain bacteria (Konkolewska et al. 2020); Pb, Ni, Cd and Cr (Alimardan et al. 2016); Cd and Pb (Alaboudi et al. 2018). Even several reports were showing that several types of heavy metals were found in marine sponges, for example, Pb, Cd, Zn, Fe and Cr (Wibowo et al. 2019; Siahaya et al. 2013; Melawaty et al. 2014), where the sponge showed a reasonable growth rate. Pseudomonas bacteria isolated from marine sponges can adsorb heavy metals Cr and Mn (Marzuki 2020).

Almost all types of bacteria that breed in areas exposed to heavy metals can carry out the function of bio-adsorption. The problem that exists for the application of bacteria in the bioremediation of environmental heavy metal contaminants is how to identify and obtain bacteria that are capable and have high performance in the bio-adsorption of heavy metals, including types of heavy metals that can be absorbed. This condition demands an assessment to find out and obtain data on the type of bacteria, the level of absorption and the types of heavy metals that bacteria can adsorb. A data bank on this matter is needed to provide convenience and quick handling of areas exposed to heavy metal, both in the aquatic environment and on land or land, especially ex-mining grounds for land function recovery, for agricultural activities. Alimardan et al. 2016).

Several types of bacteria isolated from sea sponges were stated to be able to absorb several types of heavy metals, including *Bacillus sp.* strain AB353f against heavy metals Cr and Mn (Marzuki et al. 2020b; Marzuki 2020), *Bacillus pumilus* strain GLB197 *and Pseudomonas stutzeri* strain SLG510A3-8 against heavy metals Cr(VI) and Cd(II) (Marzuki 2020). Types of bacterial isolates without known species that are symbiotic sponges have been reported to absorb several types of heavy metals. It is including *Petrosia (Strongylo Phore) Corticata*, *Neopetrosia sp*., *Callyspongia aerizusa*, *Niphates Sp*, *Hyrtios erectus* and *Auletta sp.* to heavy metal Nickel (Ni), Copper (Cu), Lead (Pb), Chromium (Cr) and Mercury (Hg) (Angela and Marzuki, 2021; Wibowo et al. 2019).

The general method of tracing bacteria has the function of bio-adsorption of heavy metals: first, identification of marine sponges that live in waters exposed to heavy metals. Second, sampling several sponges and isolate the associated bacteria. Third, the isolates obtained were tested for their bio-adsorption ability on certain heavy metals by placing some bacterial colonies on media engineered to be contaminated with several types of heavy metals, incubating and observing the growth activity of the isolates. Fourth, potential isolates from possible test results were identified and phenotype and genotype characterization. Fifth, make stock, catalogue of isolates related to types of heavy metals that can be absorbed (Gebregewergis 2020).

Researchers have carried out trials of stock bacteria that have been characterized against certain metals to see their bio-adsorption ability and performance in an engineered environment contaminated with several heavy metals in known concentrations. During the contact process between bacterial suspensions and heavy metals in an engineering environment, bacterial cell growth was observed by measuring the optical density of the interaction medium based on the contact time in days.

Experiment 10.3: Bio-adsorption of Heavy Metals Using Marine Sponge Symbiotic Bacteria

The Bio-adsorption ability of marine sponge symbiotic bacteria to heavy metal as a sewage contaminant can be determined by several procedures. First, waste is made contaminated with heavy metals, for example, Cr (III), Cr (VI), Mn (II) and Mn (VII), each with a concentration of 250 ppm and a volume of 1000 mL. Second, determine isolates of marine sponge symbiotic bacteria with bio-adsorption potential. For example, *Bacillus licheniformis* strain ATCC 9789 (BS) and *Acinetobacter calcoaceticus* strain PHKD B14 (AC), both types of bacteria was cultured on NA media, then incubated 1 × 24 O'clock. Each culture was suspended in a solution of 1,000 mL of physiological 0.9% NaCl solution. Prepare 10 reactors, each filled with 100 mL of isolate suspension, adapted for 1 × 24 h in a new environment, (3) each reactor is added 10 mL of heavy metal contaminants, such as Cr (III) or Cr (VI). The reactor is in the Shaker incubator at 150 rpm. Observation of bacterial cell growth (optical density) was carried out every 3 days. The Observation used a spectrophotometer and determined the level of bio-adsorption of heavy metals by measuring media absorption using AAS, then plotted in Eqs. (10.1) and (10.2) to determine the capacity and efficiency of bio-adsorption (Marzuki 2020).

$$\mathrm{Q} = \frac{C1 - C2}{C1} x\ V \tag{10.1}$$

$$\%E = \frac{C1 - C2}{C1} \times 100\%; \tag{10.2}$$

Based on Experiment 10.3 that has been carried out, it was obtained an overview of the growth of BC and AC bacterial cells in media contaminated with Cr (III), Cr (VI), Mn (II) and Mn (VII).

The experimental results showed that the growth of BS and AC bacterial cells was more dominant in media exposed to Cr(III) than in media contaminated with Cr (VI) (Fig. 10.8). The growth of BS cells in Cr(III) and Cr(VI) contaminated media was maximum during the first 3 days of contact and relatively low after 6 days of contact onwards (Fig. 10.8a). The growth of AC bacteria cells in media exposed to Cr (III) and Cr (VI) was relatively the same as BS bacteria. Only the first 3 and 6 days of contact in Cr (III) media were much more dominant than in Cr (VI) media which sloped from childhood. First 3 days of contact (Fig. 10.8b). The difference in the growth rate of bacterial cells in these two types of media is influenced by the ability

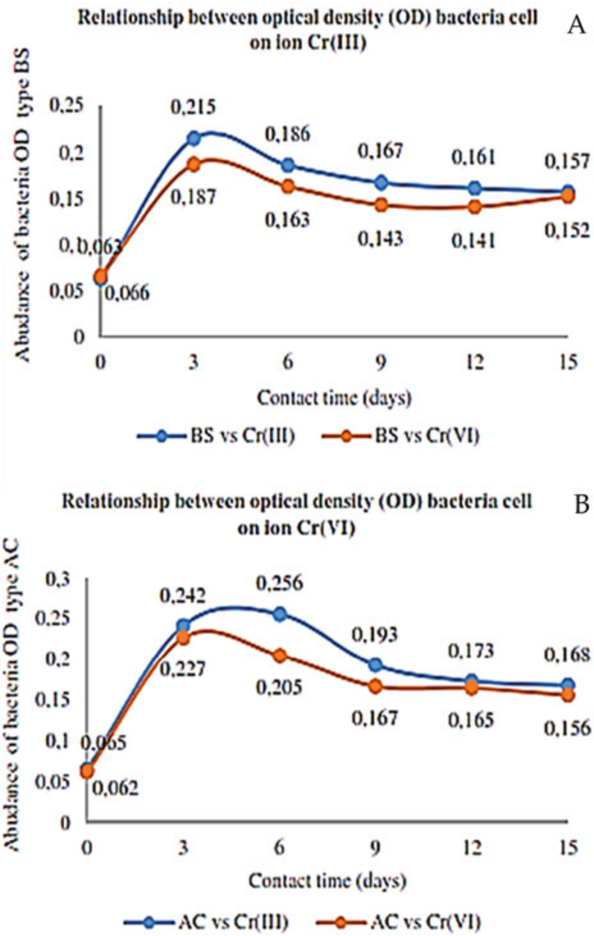
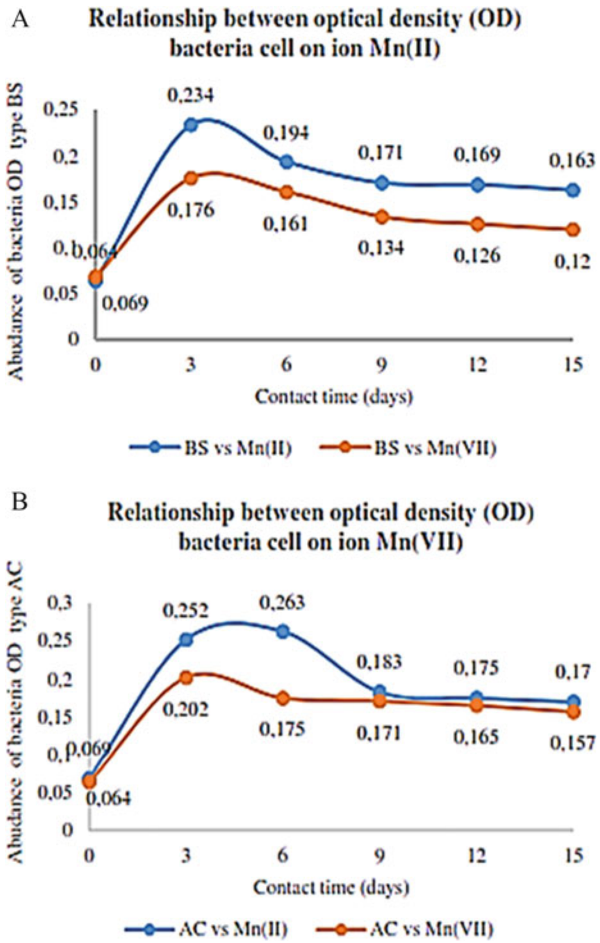


Fig. 10.8 (**a**) Growth of BS bacterial cells in Cr (III) and Cr (VI) contaminated media. (**b**) AC bacterial cell growth in Cr(III) and Cr (VI) contaminated media

of the bacterial cells to adapt to media with different levels of toxicity. It was seen that both types of bacteria BS and AC were relatively underdeveloped in media that had a higher level of toxicity (Marzuki et al. 2021b).

The treatment was the same, and the types of symbiotic bacteria were also the same. Still, Mn (II) and Mn (VII) replaced the types of heavy metal contaminants, showing that the growth of BS and AC bacteria cells did not change significantly. The growth of BS bacteria in Mn (II) media was more dominant in a wider range than in Mn (VII) media. However, the maximum growth of both types of bacteria occurred during the first 3 days of contact (Fig. 10.9a). While the dominant growth of AC bacteria cells in Mn (II) media happened at a more extended contact period, namely on days 3–6 compared to Mn (VII) media and the following contact period, the growth of AC bacterial cells in Mn (II) and Mn (VII) media was relatively the same and slopes (Fig. 10.9b) (Marzuki 2020).

Fig. 10.9 (**a**) Growth of BS bacterial cells in Mn (II) and Mn (VII) contaminated media. (**b**) Growth of AC bacterial cells in Mn (II) and Mn (VII) contaminated media



The bio-adsorption capacity was calculated using Eq. (10.1), while the bio-adsorption efficiency was determined using eq. 2, based on absorption data measured using Atomic Absorption Spectrophotometer (AAS). The bio-adsorption capacity of BS and AC bacteria to heavy metal contaminants Cr (VI) was relatively the same, as was the bio-adsorption efficiency shown by the two types of bacteria (Fig. 10.10a, b). Still, in the same picture, the curved line of bio-adsorption capacity (blue colour) does not coincide with the curve line of bio-adsorption efficiency (orange). These conditions indicate that the bio-adsorption performance of BS and AC bacteria against toxic heavy metals Cr (VI) does not reach 50%; Cr (VI) contaminants were chosen because the toxicity level is higher than Cr (III) (Marzuki 2020).

The bio-adsorption capacity of BS and AC bacteria against heavy metal contaminants Mn (II) was relatively the same, including the bio-adsorption efficiency

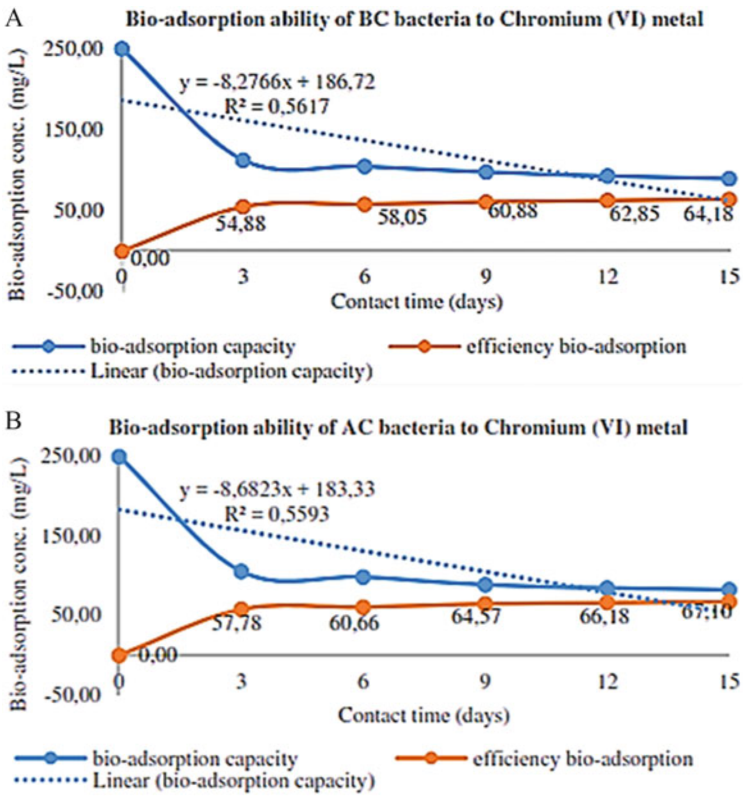


Fig. 10.10 (**a**) Bio-adsorption capacity and efficiency of BS bacteria against Cr(VI) contaminants. (**b**) AC bacteria bio-adsorption capacity and efficiency against Cr(VI) contaminants

performance shown by the two types of bacteria (Fig. 10.11a, b). What is different from the bio-adsorption version of these two types of symbiotic sponge bacteria is the performance of the bio-adsorption capacity shown against heavy metal contaminants Mn (II) exceeds 50%. The two types of bacteria used as bio-adsorbents for heavy metals are Bacteria Bacillus sp. AB353f partial (BS) is an isolate of the sponge *Neopetrosia sp.*, while the bacterium *Acinetobacter calcoaceticus* strain PHCDB14 (AC) is an isolate of the marine sponge *Callyspongia aerizusa* (Marzuki et al. 2021b).

The performance and level of bio-adsorption of heavy metals by symbiotic bacteria of marine sponges have not shown maximum achievement in environmental remediation. So it is necessary to make efforts and developments to obtain bio-adsorption results of heavy metals bio-adsorption capacity reaching 75–90%. The approach that can be taken to improve the bio-adsorption performance of heavy metals is by tracing the types of bacteria that have high bio-adsorption performance against heavy metals. A consortium of bacterial cells that can bio-adsorb heavy

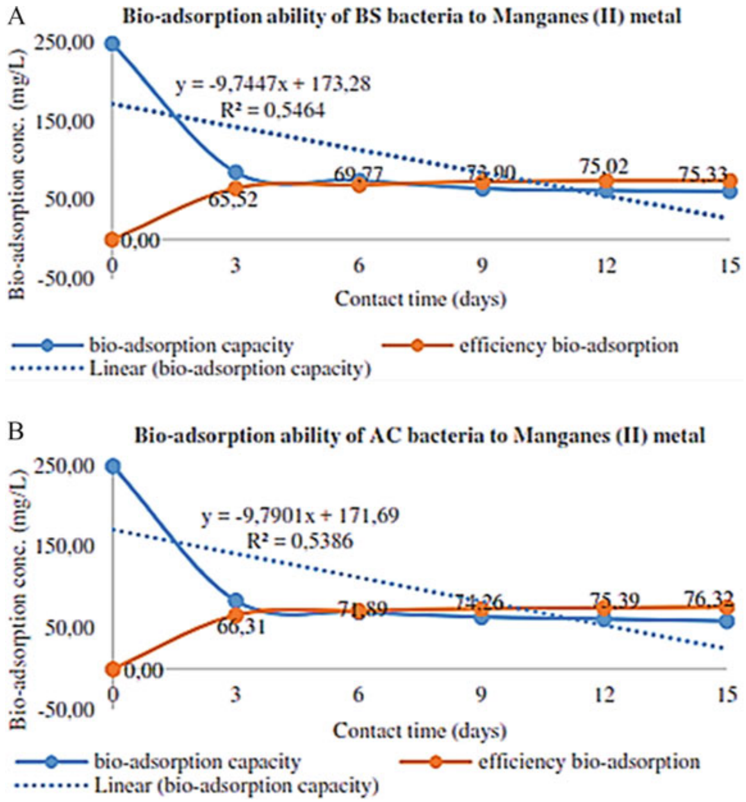


Fig. 10.11 (**a**) Bio-adsorption capacity and efficiency of BS bacteria against Mn(II) contaminant. (**b**) AC bacteria bio-adsorption capacity and efficiency against Mn(II) contaminants

metals; waste engineering and modelling interactions between bacterial suspensions and heavy metal contaminants, interaction time, bacterial nutrient supply; and other modifications aimed at improving the bio-adsorption performance of heavy metals by bacteria (Orani et al. 2018).

Efforts to improve the bio-adsorption performance of sponge symbiotic bacterial cells against heavy metals, especially the use of consortium bacteria, are currently underway by our research team. But, the researcher cannot publish the results and achievements of the bio-adsorption obtained because they are still in the experimental stage and process. Nevertheless, we offer recommendations for the method that we are currently running and the detailed procedure in Experiment 10.4. An overview of the development of heavy metal bio-adsorption methods using several types of heavy metal bio-adsorbent bacteria for utilization in waste exposed to several kinds of heavy metals aims to improve performance maximal bio-adsorption and bioremediation on various types of heavy metal contaminants in parallel (Sobrinho et al. 2020).

Experiment 10.4: Application of Marine Sponge Symbiotic Bacteria Consortium Against Heavy Metal Extreme Waste

The heavy metal bio-adsorption procedure using a consortium of sponge symbiotic bacteria isolates was carried out in several stages. (1) Manufacture of artificial waste contaminated with several types of heavy metals (Pb, Hg, As, Cr and Cu). Each type of heavy metal made a solution of each concentration of 1000 ppm as much as 1000 mL. The heavy metal solution is mixed in a sizeable homogenized portion (extreme waste). (2) Determine five types of potential sponge symbiotic bacterial isolates, the cells of each selected isolate were propagated by culture and incubated for 1×24 h. The cultured isolates were suspended in a 1000 mL solution containing physiological 0.9% NaCl. (3) The five types of mixed isolate suspension in one large container, homogenized (consortium bacteria). (4) Prepare ten reactors, each filled with 500 mL of bacterial suspension of the consortium, adapted for 1 x 24 hours in an incubator. (5) Each reactor is put in 100 mL of extreme waste and a shaker incubator at 200 rpm. (6) Observations and measurements of bacterial cell growth were carried out every 2 days, and measurements of the absorption of each type of heavy metal were to determine the bio-adsorption achievement of the consortium bacteria based on the contact period.

Other developments related to the exploration of the dual function of sponge symbiotic bacterial cells in the biodegradation of PAHs as well as the bio-adsorption function of heavy metals can be carried out by carrying out a similar procedure in Experiment 10.4 above, but with differences in the type of waste and the bacterial consortium used. The engineering waste is made to be super extreme. In addition to containing PAHs contaminants (the type and concentration of PAHs are known), the waste also includes several types of heavy metals (the type and concentration of heavy metals are known). This super extreme waste is homogenized (Bertollino 2019). This extreme super waste modification resembles petroleum waste, which contains several types of heavy metals and containing PAHs. The bacterial consortium was made by having ± six types of symbiotic sponge bacteria, a combination of three types of bacteria that can biodegrade and three types of bacteria with the ability to bio-adsorb heavy metals. The selected sponge symbiotic bacterial cells were multiplied by the culture method, made a suspension, mixed in one container, homogenized, and adapted first. This dual-function bacterial consortium suspension is ready to have interacted with super extreme waste (Wibowo et al. 2019).

The parameters observed for this method include the optical density of bacterial cell growth, changes in pH values, shifts in interaction temperature values, whether there is the formation of gas bubbles in the interaction medium, and whether there is the formation of a fermentation odour. At the same time, the instrumentation recommended for use is GC–MS, FT–IR, and SSA. GC–MS is an instrument in determining the biodegradation process by looking at the abundance of PAHs, the types of simple organic compounds as biodegradation products. FT–IR is an instrument to assess the kind of biodegradation product, whether the alcohol group is an aldehyde, ketode, carboxylic acid and other organic components. In comparison, SSA is an instrument to determine the bio-adsorption capacity that occurs by determining the uptake of each heavy metal present in the extreme super waste and compared with the initial concentration (Ziarati et al. 2019b).

Bioremediation methods to change and produce friendly environmental quality are the dream of every citizen on this earth. Still, this dream is sometimes difficult to realize because, directly or indirectly, at the same time, we produce by-products, by-products and wastes that are pressing environment so that the environment is massively under continuous pressure and at its natural balance point (Sabdono and Radjasa 2008). Nature provides biomaterials that have a role and function in environmental bioremediation, one of which is sea sponges that can form a symbiosis with various types of bacteria. Symbiotic sponge bacteria have a biodegradation function against PAH components. It includes a heavy metal bio-adsorption function and a strong suspicion that several types of symbiotic sponge bacteria have dual biodegradation and bio-adsorption functions. A collection of several types of symbiotic marine sponge bacteria that have the same role in both the biodegradation of PAHs and the bio-adsorption of heavy metals is called a bacteria consortium (Parhamfar et al. 2020). The term for the type of bacteria with the ability to biodegrade hydrocarbons is known as carbonoclastic bacteria. The type of bacteria that has the capacity for bio-adsorption of heavy metals is metallo clastic bacteria. The term for the symbiotic bacteria of marine sponges can biodegrade hydrocarbons and bio-adsorption of heavy metals called metallo-carbonoclastic bacteria (Marzuki et al. 2021b).

The role and contribution of marine sponges in environmental bioremediation are evident. Including the content of secondary metabolite components possessed by marine sponges have an essential role in biodegradation and bio-adsorption, including the potential utilization of sponge secondary metabolites as biomaterials in overcoming exposure to bioplastics and primary materials in the manufacture of drugs and various other functional potentials. The potentials inherent in sponges, so that marine sponges are recommended for screening to explore potential and develop utilization for the common good of humans and the environment (Saputra et al. 2019). One of the recommendations for marine sponge population is those development efforts to maintain and increase the sponge population in their habitats. Keeping the population of marine sponges can be carried out through the transplant method. Especially the type of marine sponge has been confirmed to have symbiosis against several bacteria in bioremediation and sponges that have been identified as capable of producing chemical components bioactive (Bibi et al. 2016).

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